

PHYSICO-CHEMICAL CHANGES ON PUMPKIN/PEPPER SAUCES DURING REFRIGERATED STORAGE

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Accepted for Publication October 3, 2011

doi:10.1111/j.1745-4549.2011.00644.x

ABSTRACT

Changes in rheological, textural and physicochemical characteristics of pumpkin and red bell pepper sauces prepared in 50:50 and 80:20 proportions were studied for 6 days under refrigerated conditions. Variations in sugar, total carotenoids and antioxidant capacity were determined. The compression forces of sauces decreased by 50% at eighth day of storage and sauces behaved as elastic solids with G' values predominating over G'' values. The storage modulus was less frequency dependent than the loss modulus, giving values of $\tan \delta$ between 0.1 and 0.2, which tends to indicate a weak gel system. No significant changes were observed in the physicochemical parameters, except total antioxidant capacity, which decreased by 50% after 6 days. Microbiological counts showed slight increases or remained invariable in total counts for mesophylic bacteria, yeasts and molds during storage.

PRACTICAL APPLICATIONS

The effect of storage time at 4C on textural and rheological behavior, total sugar, total carotenoids and antioxidant capacity of the pumpkin/pepper sauces in 50:50 and 80:20 proportions was studied. Besides, microbiological control was done. The practical application of this study is the formulation of a stable mix of pumpkin and pepper in the form of a sauce that could be used as seasoning in meat and other dishes.

INTRODUCTION

Pumpkins and red bell peppers play a significant role in providing nutrients and vitamins to the population of the world. Pumpkin is a good source of micronutrients such as carotenoids, potassium, and vitamins B₂, C and E, with low caloric content and good quantities of fiber (de Escalada Pla *et al.* 2007). Red bell pepper is a very popular fruit, not only for its attractive color, taste and aroma, but also for its beneficial health properties because of its bioactive component contents. Bioactive compounds are substances found in food which are recognized for having health benefits; and that is the reason why an increase in consumption of these kinds of substances is recommended worldwide (OMS 2006). Among the main bioactive compounds, we could mention the carotenoids, tocopherols, polyphenolics, ascorbic acid, and so on (Larson 1977), which can prevent or diminish the develop-

ment of certain kinds of cancer, cardiovascular diseases and macular degeneration which is mainly attributed to the antioxidant capacity of these compounds (Hollman *et al.* 1996; Ishida and Chapman 2004; Rao and Rao 2007). β -Carotene, α -carotene and lutein have been isolated in pumpkins (González *et al.* 2001; Murkovic *et al.* 2002; Azevedo-Meleiro and Rodriguez-Amaya 2007) and in peppers, high levels of antioxidant substances such as ascorbic acid and carotenoids (capsanthin, β -carotene, violaxanthin, cryptoxanthin, capsorubin and luteolin) have been found (Hornero-Méndez and Mínguez-Mosquera 2001; Collera-Zúñiga *et al.* 2005; Sun *et al.* 2007).

The pumpkin is consumed in small pieces, once cooked, and used in derived foods such as sauces, marmalades, sweets and purées (Gliemmo *et al.* 2008). The pepper is consumed cooked or fresh, in salads or juices mixed with other fruits or vegetables (Chuah *et al.* 2008). The formulation of a mix of

both vegetables in the form of a sauce, or mashed and used as seasoning in meat, sandwiches, pastas and other dishes, could provide important nutrients and a contribution of bioactive compounds to many food preparations, as well as benefit regional economic development.

There is currently no information about the physical and chemical changes that sauces made of pumpkin and red pepper undergo, although Bayod *et al.* (2008) found a correlation between the structural and rheological aspects of tomato pastes and processed ketchups, as well as the sensory perception of the sauces. Ahmed *et al.* (2002) reported the variations in color of paste in red chilli peppers during thermal treatment and Dutta *et al.* (2006) the effects on rheological properties of pumpkin purée. Gliemmo *et al.* (2008) studied the effect of storage on the stability of color in pumpkin purée (*Cucurbita moschata*, Duchesne ex Poirer), and there are studies about changes on carotenoids and the antioxidant capacity of other vegetables stored under refrigeration (Choi *et al.* 2002; Piljac-Žegarac *et al.* 2008; Ordóñez-Santos *et al.* 2009). In mango purée/nectar, Vásquez-Cañedo *et al.* (2007) observed changes in color, acidity, flow properties and β -carotene stability during thermal treatment. It must be taken into account that the quality attributes of sauces and purées are their organoleptic (color, flavor and texture), nutritional and microbiological characteristics. Particularly, texture and rheological behavior are of critical importance for consumer acceptability, as well as for packaging system selection.

The purpose of this study is to investigate physicochemical, nutritional and microbiological characteristics of sauces/purées made of pumpkin and red bell pepper at a storage temperature of 4C.

MATERIALS AND METHODS

Raw Material

The experiments were carried out with pumpkin (*C. moschata*, D.) and red bell pepper (*Capsicum annuum*, L.), both purchased at a wholesale market in Corrientes (Argentina), and 10 kg of each was used for each assay.

Sauce Preparation and Storage

Before cooking, the fresh pumpkins were washed under tap water, hand-peeled, deseeded, sanitized with hypochlorite (10 ppm/3 min), rinsed and sliced (15–20 mm thick). Then, they were steamed for 10 min in a steam oven. Peppers were wrapped in aluminum foil and put into an oven at 180C for 60 min. Then, their skin was removed and finally they were washed with distilled water. The edible tissues were mashed and homogenized using a kitchen food processing machine. Sauces were prepared in four proportions of pumpkin : pep-

per purée (80:20, 70:30, 60:40 and 50:50). The more acceptable formulation was choosing by an acceptability test. In this way, sensory attributes (general appearance and color) of all samples were evaluated by 60 consumers. They gave a score for each sample with respect to their perceptions of each sensory attribute. 80:20 and 50:50 pumpkin : pepper proportion was found to be best with respect to their appearance scores by 90% of the consumers. However, the color of 50:50 formulations was accepted by 65% of them. Then, the sauces were placed in PET containers of 200 cm³ each and 25 containers of each proportion were used for the storage assays. Sauces were stored under refrigeration at 4 ± 2C for 6 days and five containers were carried out every 2 days and repeated twice. The end of the experiments was set by quality loss which was determined by textural changes, leakage of juice and off-flavors.

Texture Determination

Back extrusion assays were carried out using Stable Micro System's TA.XT2 texture analyzer with an extrusion cell of 48 mm × 74 mm with a 45-mm-diameter acrylic piston at a penetration of 20 mm. The selected parameters during assays were pre-assay speed of 2.5 mm/s, assay speed of 1 mm/s, applied force of 0.5 N and measurement temperature of 4C. The software of Texture Expert Stable Micro Systems Ltd. (1995) was used for data analysis.

Packing (F_1), compression (F_2) and rupture (F_3) forces were registered (Fig. 1). Packing force was determined by the slope of the initial nonlinear part of the back extrusion curve and represents the packing of the sample into the test cell. In the same way, compression force is the slope of the linear part of the curve and it is related to the apparent elastic properties of

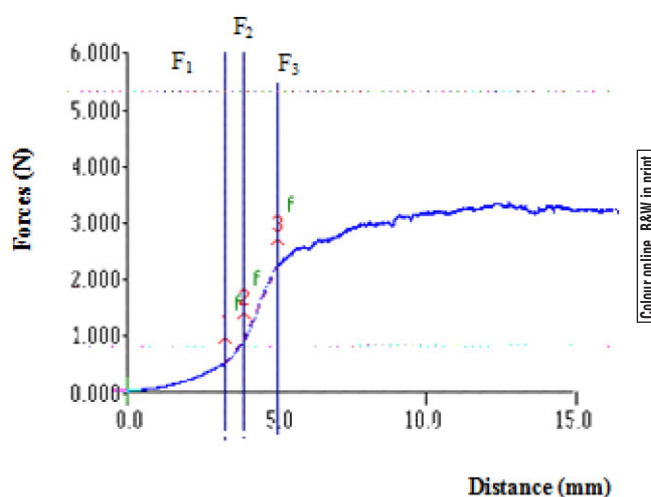


FIG. 1. FORCE-DISTANCE CURVE OBTAINED WITH THE BACK-EXTRUSION TEST ON 50:50 PUMPKIN : PEPPER SAUCES

1 the sample. Rupture force (F_3) was determined by the abrupt
2 change of slope when the sample begins to rupture or shear
3 (Bourne 1982). These parameters were measured until eighth
4 storage day when the sauces show juice leakage.

6 **Dynamic Rheological Measurements**

7 Dynamic rheological measurements of sauce samples were
8 carried out in a controlled-stress rheometer HAAKE RS600,
9 with a 35-mm-diameter Titanium C35/2 cone-plate sensor
10 system at a 2° angle and a MP60 18/8 steel plate. Data were
11 obtained using RheoWin's JobManager software. A stress
12 sweep at a range of 0.01–50 Pa was carried out in order to
13 determine the range of linear viscoelastic response under
14 oscillatory shear conditions, under a constant frequency of
15 1 Hz. Then a frequency sweep was carried out, at a range of
16 0.01–100 Hz, with constant stress amplitude of 10 Pa. Mea-
17 surements were performed at a constant temperature of 25C.
18 The following parameters were obtained: storage modulus
19 (G'), lost modulus (G'') and $\tan \delta$ as a function of
20 frequency.

22 **Water Content, pH and Titratable Acidity**

23 Water content of the samples was determined by using a
24 vacuum oven at 60C (AOAC 1990) until constant weight of
25 the samples.

26 The measurement of pH was carried out with a solid elec-
27 trode Hach pH meter in five different positions. Titratable
28 acidity was quantified with 0.083 N NaOH until the pH
29 reached 8.1.

31 **Total Sugar**

32 Total sugars were extracted with 80% ethanol and the
33 anthrone method was used for quantification (Southgate
34 1976). A 10-g sample was homogenized with ethanol by
35 adding 1,000 μ L of ethanolic extract to 4 mL of 0.5%
36 anthrone solution, boiled for 15 min and left to cool for
37 30 min before the reaction product was read spectropho-
38 tometrically at 620 nm. Calibration curves were prepared with
39 **4** glucose solution between 25 and 200 μ g/mL and results were
40 given as milligram of glucose per gram of sample.

42 **Antioxidant Capacity**

43 Antioxidant capacity was determined by spectrophotometry,
44 measuring the loss of color of the radical chromogen DPPH•
45 (Brand-Williams *et al.* 1995). A sample of 10 g was thor-
46 oughly mixed with cold methanol. After 15 min, this was fil-
47 tered and diluted successively with cold methanol until
48 complete discoloration of the residue. A 6 mL aliquot of
49 methanolic DPPH• solution (3×10^{-3} mM) was added to

500 μ L of the obtained methanol extract and left for 20 min
in complete darkness to reach a stationary state. Then a
reading of the solution was taken to monitor the decrease in
its absorbance at 517 nm. The antioxidant capacity was
then expressed as its equivalent in ascorbic acid, for which
standard solutions of ascorbic acid ranging from 0.5 to
2.5 mg/mL were utilized.

58 **Total Carotenoids**

59 Frozen samples (10 g each) were crushed in a mortar with
60 20 mL of cold acetone (5C) and vacuum filtered through a
61 Buchner funnel fitted with a 10-cm-diameter No 1 filter paper
62 (Whatman International Ltd., Maidstone, England) until
63 total discoloration. Two extracts were done for each storage
64 time. Then the pigments were extracted by petroleum ether
65 (b.p. 40–60°) and absorbance was read at 450 nm in the ether
66 phase and results were expressed as μ g β -carotene/g. (Davies
67 *et al.* 1970).

69 **Microbiological Determination**

70 A sample (10 g) was aseptically transferred to a vessel, diluted
71 to a ratio of 1:9 in peptone physiological salt solution (0.1 g/
72 100 mL bacteriological peptone) and homogenized by mac-
73 eration for 1 min. The subsequent 10-fold dilution was also
74 made in peptone. Total counts (NOM-092-SSA1-1994) and **5**
75 yeast and mold counts (NOM-111-SSA1-1994) were per- **6**
76 formed by the pour plate method using 1 mL of inoculum.

77 All determinations were performed in duplicate.

79 **Statistical Analysis**

80 The experiment was performed with a factorial design in
81 sauce formulation and storage time (2×4) and was repeated
82 twice. Data were treated by two-way analysis of variance and
83 statistically significant differences between means were deter-
84 mined by the least significant difference test ($\alpha = 0.05$) by the
85 software INFOSTAT/Professional 1.1.

87 **RESULTS AND DISCUSSION**

89 **Physicochemical and Nutritional Determinations**

90 pH and titratable acidity values of 80:20 and 50:50 sauces are
91 given in Table 1 and there are no significant differences
92 between them ($P > 0.05$). The sauces have higher pH values
93 and low acidity than those reported for other varieties of
94 pumpkin and pepper fruits used to prepare individual purées
95 (Bozkurt and Erkmen 2004; Dutta *et al.* 2006).

96 Moisture levels of 50:50 pumpkin : pepper sauces were
97 higher than 80:20 proportion as well as the pigments, total
98

TABLE 1. PHYSICOCHEMICAL PARAMETERS IN 80:20 AND 50:50 SAUCES BEFORE STORAGE

| Sauces | pH | Acidity (% citric acid) | Water content (%) | Total sugar (mg glucose/g) | Total carotenoids ($\mu\text{g } \beta\text{-carotene/g}$) | Starch content (mg glucose/g) | Antioxidant capacity (mg ascorbic acid/g) |
|--------|------------------|----------------------------|----------------------|-------------------------------|---|----------------------------------|--|
| 80:20 | 5.82 \pm 0.01a | 0.16 \pm 0.008a | 89.60 \pm 0.64a | 74.34 \pm 1.63a | 50.30 \pm 3.02a | 52.90 \pm 3.61b | 1.62 \pm 0.02a |
| 50:50 | 5.76 \pm 0.01a | 0.15 \pm 0.003a | 91.16 \pm 0.20b | 77.64 \pm 0.04b | 60.00 \pm 0.31b | 27.62 \pm 4.87a | 2.82 \pm 0.05b |

a, b values indicate significant differences ($P < 0.05$), within columns.
 Main value \pm standard deviation, $n = 4$.

TABLE 2. TOTAL SUGAR, TOTAL CAROTENOIDS AND ASCORBIC ACID VARIATION IN 80:20 AND 50:50 SAUCES DURING STORAGE AT 4C

| Sauces | Total sugar (mg glucose/g) | | | | Antioxidant capacity (mg ascorbic acid/g) | | | |
|--------|----------------------------|--------------------|--------------------|--------------------|---|--------------------|--------------------|--------------------|
| | 0 | 2 | 4 | 6 | 0 | 2 | 4 | 6 |
| 80:20 | 50.30 \pm 3.02ax | 47.63 \pm 0.74ax | 45.60 \pm 1.12ax | 46.74 \pm 1.23ax | 75.24 \pm 1.63ax | 74.34 \pm 1.63ax | 63.83 \pm 2.29ay | 64.02 \pm 0.83ay |
| 50:50 | 60.00 \pm 0.31bx | 60.21 \pm 0.65bx | 60.90 \pm 0.42bx | 59.50 \pm 0.59bx | 76.44 \pm 0.04bx | 77.64 \pm 0.11ax | 67.01 \pm 2.34ay | 54.29 \pm 2.06ay |

Main value \pm standard deviation, $n = 4$.
 a, b values indicate significant differences ($P < 0.05$), within columns.
 x, y, z values indicate significant differences ($P < 0.05$), within rows.

sugar and antioxidant capacity. These differences can be attributed to higher amount of red pepper in its formulation (Berhardt and Schilch 2006; Dutta *et al.* 2006; Chuah *et al.* 2008). On the other hand, the starch levels were superior in sauces with a greater percentage of pumpkin (Table 1).

During the storage periods, the acidity, pH and water level of 80:20 sauces were invariable (data not shown), while total sugar decreased 14.1% at day 4 and remained invariable at the end of the storage (Table 2). Starch levels were between 52.90 \pm 3.61 and 49.10 \pm 1.44 mg glucose/g during the storage ($P > 0.05$).

Likewise, in 50:50 sauces there was no evidence of changes in pH, acidity and water levels ($P > 0.05$) with similar values to those obtained in 80:20 sauces. According to the total sugar levels, it was similar behavior to 80:20 sauces and showing a significant drop at fourth and sixth days (Table 2). The initial starch level was 27.62 \pm 4.87 mg glucose/g and it was invariable during storage period ($P > 0.05$).

Total carotenoid levels of both sauces were constant until sixth storage day probably due to the inactivation of the enzymes involved in their damage during steam exposure or oven treatment, although the stability of carotenoids could be affected by storage, temperature, light, presence of oxygen, antioxidant compounds, pH, as well as the previous treatments given to the product (Rodríguez-Amaya 1999). Other authors have reported that the carotenoids in bottled tomato pulp stored at 20C remained stable during 180 days (Kuc *et al.* 2005; Ordóñez-Santos *et al.* 2009), whereas for orange juice, the carotenoids were reduced by 6.6% at 49 days during refrigerated storage (Choi *et al.* 2002).

The antioxidant capacity of a product is the result of a contribution made by the individual compounds, in addition to the synergistic and antagonistic effects between them and the food matrix. The principal antioxidant compounds in the assayed samples are carotenoids, polyphenols and ascorbic acid, which all contribute to the total antioxidant capacity. During storage, a significant drop in antioxidant activity ($P < 0.05$) at fourth day was observed (Table 2), showing similar trends for both assayed proportions. Similarly, in heat-treated bottled tomatoes a mean loss of 34% in the total antioxidant activity after 7 days of storage has been measured (Kuc *et al.* 2005), and in dark fruit juices, Piljac-Žegarac *et al.* (2008) have found significant losses in antiradical activity during storage at 29 days (Ordóñez-Santos *et al.* 2009).

Texture

The results from back extrusion test are shown in Fig. 2A,B. Textural parameters of the 80:20 and 50:50 pumpkin : pepper sauces were unchanged until sixth storage day. The packing force (F_1) diminished significantly at the eighth storage day for the sauces in both assayed proportions ($P < 0.05$). Additionally, the compression force (F_2) presented lower values at

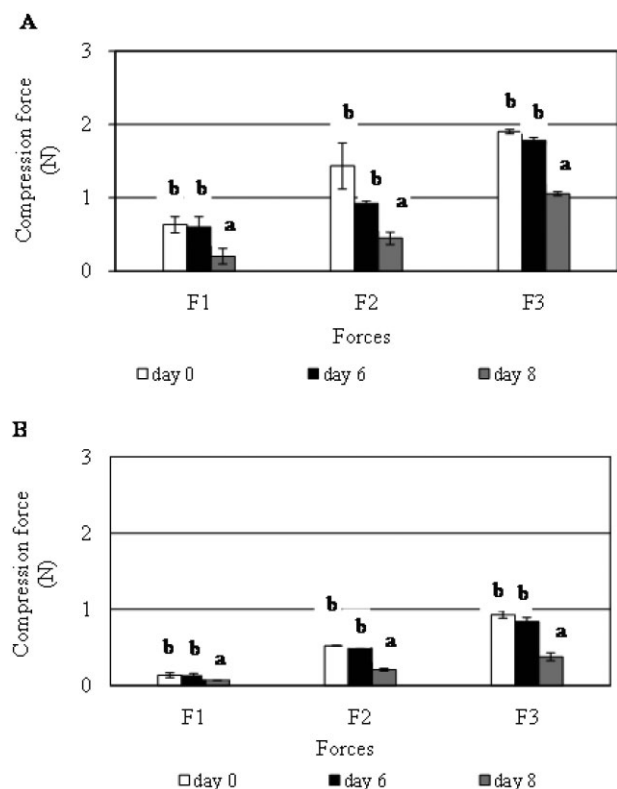


FIG. 2. EFFECTS OF STORAGE TIME ON COMPRESSION FORCES OF PUMPKIN : PEPPER SAUCES (A) 80:20 (B) 50:50
a, b values indicate significant differences ($P < 0.05$) within storage day.

the final assay time, as the rupture force did (F_3), and registered before the breaking of the samples. Significant variations in all forces were registered ($P < 0.05$) at eighth storage day. Besides, it must be noticed that force values for the 80:20 sauces were, at least, two times higher than in 50:50 sauces and showed less deformation because of greater pumpkin content. This can be attributed mainly to the higher starch content, as well as to other minor constituents (Loy 2006). Total solids of pumpkin are closely 1% higher than in pepper (FAO 2009) and these strongly influence the textural characteristics (Brecht *et al.* 1995).

Rheological Behavior

The mechanical spectra of sauces in the linear viscoelastic range at the beginning of storage period show a solid-like

TABLE 4. DYNAMIC RHEOLOGICAL PARAMETERS AT 1 Hz IN 50:50 SAUCES DURING STORAGE AT 4°C

| Storage (days) | G' (Pa) | G'' (Pa) | $\tan \delta$ |
|----------------|------------------------|--------------------|--------------------|
| 0 | 1,164.0 \pm 36.7a | 267.2 \pm 115.0a | 0.169 \pm 0.008a |
| 2 | 2,083.5 \pm 210.0a | 400.3 \pm 131.8a | 0.190 \pm 0.040a |
| 4 | 2,763.5 \pm 1,021.7a | 531.8 \pm 224.5a | 0.190 \pm 0.010a |
| 6 | 1,214.0 \pm 56.8a | 268.1 \pm 152.3a | 0.217 \pm 0.020a |

Main value \pm standard deviation, $n = 4$.

a, b values indicate significant differences ($P < 0.05$), within columns.

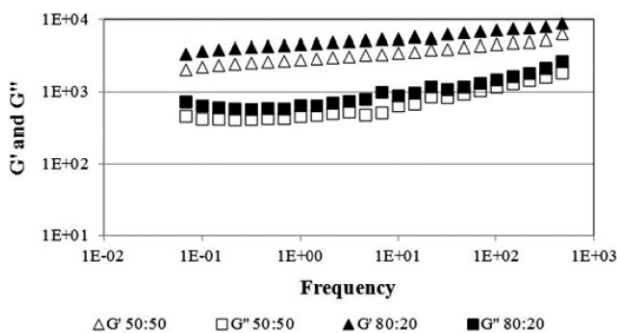


FIG. 3. G' AND G'' VARIATION WITH FREQUENCY IN 80:20 AND 50:50 PUMPKIN : PEPPER SAUCES BEFORE STORAGE AT 4°C

elastic behavior because elastic modulus G' is over the viscous modulus G'' in all frequency range. It was also observed that both moduli increased with frequency (Fig. 3). Bayod *et al.* (2008) found similar trends for G' and G'' in ketchup prepared from tomato paste.

In order to analyze the viscoelastic behavior of the sauces, G' , G'' and $\tan \delta$ -values were registered at a frequency of 1 Hz (Table 3). G' magnitudes of 80:20 sauces were higher than 50:50 ones, indicating more elasticity ($P < 0.05$). According to other parameters, not significant differences were found between sauces ($P > 0.05$).

During storage period, the rheological behavior of the 50:50 sauces was studied because they were the most preferred organoleptically.

The variation of G' , G'' and a $\tan \delta$ -value is presented in Table 4. There was a slight increase in G' and G'' for 4 days and a slight decrease thereafter, although these changes were not significant ($P > 0.05$), while the values of $\tan \delta$ did not show a significant variation ($P > 0.05$) and remained higher than 0.1.

Linear regression plots of $\log G'$ and $\log G''$ against \log frequency of sauces were generated (Fig. 4). From these dynamic

TABLE 3. DYNAMIC RHEOLOGICAL PARAMETERS AT 1 Hz IN 80:20 AND 50:50 SAUCES BEFORE STORAGE

| Sauces | G' (Pa) | G'' (Pa) | $\tan \delta$ |
|--------|----------------------|-------------------|------------------|
| 80:20 | 6,670.5 \pm 194.5b | 446.5 \pm 54.4a | 0.14 \pm 0.01a |
| 50:50 | 2,837 \pm 198.7a | 455.5 \pm 23.3a | 0.16 \pm 0.02a |

Main value \pm standard deviation, $n = 4$.

a, b values indicate significant differences ($P < 0.05$), within columns.

TABLE 5. TOTAL MICROBIOLOGICAL RECOUNT OF 80:20 AND 50:50 SAUCES DURING STORAGE AT 4C

| Sauces | Mesophylic bacteria | | | | Molds and yeasts | | | |
|--------|---------------------|---------------|---------------|---------------|------------------|---------------|---------------|---------------|
| | Day 0 | Day 2 | Day 4 | Day 6 | Day 0 | Day 2 | Day 4 | Day 6 |
| 80:20 | 4.23 ± 0.12ax | 4.11 ± 0.16ax | 4.03 ± 0.06ax | 3.96 ± 0.11ax | 4.05 ± 0.07ax | 4.00 ± 0.03ax | 4.05 ± 0.08ax | 4.87 ± 0.13ay |
| 50:50 | 4.03 ± 0.04ax | 3.97 ± 0.03ax | 3.93 ± 0.05ax | 4.94 ± 0.08ay | 3.97 ± 0.03ax | 4.02 ± 0.03ax | 3.85 ± 0.16ax | 4.88 ± 0.02ay |

Main values ± standard deviation, $n = 4$ are expressed as log cfu/g.
 a, b values indicate significant differences ($P < 0.05$), within columns.

x, y, z values indicate significant differences ($P < 0.05$), within rows.

rheological data, it was found that G' and G'' are slightly frequency dependent and G'' always appeared to be more frequency dependent than G' . This rheological behavior is typical for weak gels. "Weak gels" or "physical gels" behave somewhere between "true gels," characterized by covalent cross-linked materials, and "concentrated suspensions," characterized by entanglement networks, as observed by Bayod *et al.* (2008) for tomato paste and ketchup. During storage, the overall profile of the frequency curves does not change indicating that the viscoelastic properties of the sauces were maintained well during this period.

Microbiological Determinations

Mesophylic bacteria and mold and yeast counts of sauces during the refrigerated storage period are given in Table 5.

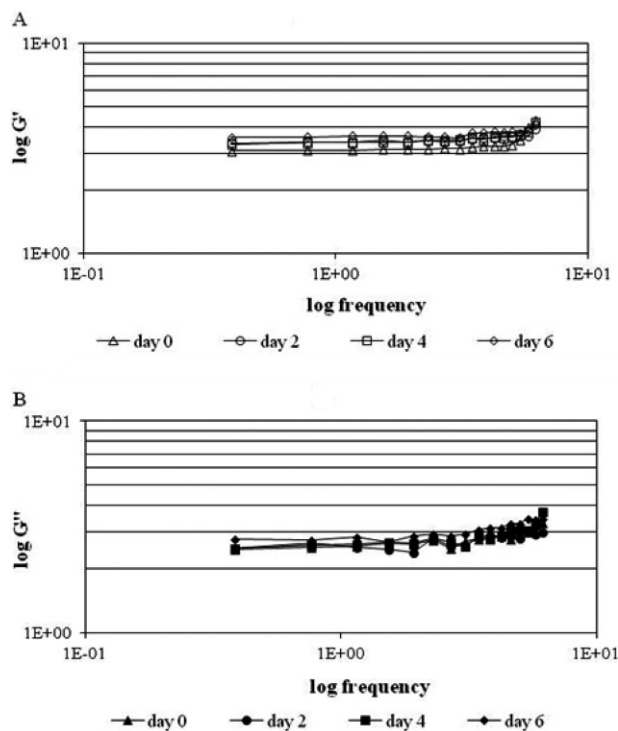


FIG. 4. (A) LOG G' AND (B) LOG G'' VERSUS LOG FREQUENCY IN 50:50 PUMPKIN: PEPPER SAUCES DURING STORAGE PERIOD AT 4C

Increments of 1 log cycle in mesophylic bacteria counts after fourth storage day were noticed in 50:50 pumpkin : pepper sauces, while it remained invariable in 80:20 sauces. However, mold and yeast counts noted an increase of 1 log cycle after fourth day. Bozkurt and Erkmen (2004) recorded the initial plate counts of 5.80 for aerobic bacteria and 4.74 for molds and yeasts in hot pepper paste, while after storage for 7 days at 37C decreased 1 and 3 log, respectively.

CONCLUSION

Data from this study suggest that during six storage days, both the 80:20 and 50:50 (pumpkin/pepper) sauces registered similar tendencies in their physicochemical, nutritional and microbiological parameters, with special attention paid to the texture parameters for observing the sauces' quality for consumption. No changes in pH, acidity and moisture content were found in either sauce during storage, while a great reduction in the antioxidant capacity was observed toward the end of the storage time. However, the content of carotenoids was constant during this period, probably due to the inactivation of enzymes during the cooking process. The 50:50 sauces showed less resistance to deformation than the 80:20 sauces. After 6 days of storage, significant decreases in compression forces were observed for both sauces. Both sauces behaved as an elastic solid with $G' > G''$ and demonstrated the structural properties of a "weak gel" throughout the entire testing period.

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






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